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# Separation of diastereoisomers of podophyllum lignans by micellar electrokinetic chromatography

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**Abstract**

Seven pairs of diastereoisomers of podophyllum lignans at 2-position, including two pairs of spin-labeled compounds, were separated by micellar electrokinetic chromatography with 5 m*M* sodium tetraborate–20 m*M* $NaH_2PO_4$–120 m*M* sodium dodecylsulfate–30% (v/v) 2-propanol (pH 6.5–7) within 35 min. The migration behaviors of the compounds with different types and concentrations of organic modifiers were studied. The method can be used to identify the purification of the lignans, monitor the 2-H configuration transformation, and determine the 2-H configuration of the spin-labeled derivatives. 

*Keywords:* Diastereomer separation; Lignans; Podophyllotoxins; Toxins

## 1. Introduction

Podophyllotoxin (1), an aryltetralin lactone, occupies a unique position among lignan natural products since its glucopyranoside derivatives such as VP-16, VM-26 and Etopofos have been widely and successfully used in the chemotherapy of cancers [1,2]. Recently, it was found that podophyllotoxin and its naturally occurring derivatives are potent inhibitors of microtubule assembly and arrest cell growth during mitosis [3]. Research of structure–activity relationships indicated that the biological activity and toxicity of podophyllotoxin and its derivatives are stereochemically specific. The base-catalyzed epimerization of the rigid structures with 2β-H configuration to the flexible structure with 2α-H configuration (Fig. 1) led to the great decrease in toxicity as well as of antimitotic activity. It is therefore important to develop an effective method for monitoring the epimerization in the isolation of natural products, chemical reactions and pharmaceutical metabolism, etc. The epimerization from 2β-H configuration to 2α-H configuration was investigated by proton nuclear magnetic resonance ($^1$H NMR) spectrometry [4]. Because pure compounds and deuterated solvent are required for the $^1$H NMR measurements, it is not convenient with the method to study a kinetic process in chemical reactions or biochemical fluids. Recently, we have synthesized some novel spin-labeled derivatives of podophyllotoxin, which have better anticancer activity and less toxicity than parent compounds [5]. The 2-H configurations of these spin-labeled derivatives cannot be determined by $^1$H NMR because of increasing line width particularly in paramagnetic molecules [6]. Hence it is also an urgent subject to

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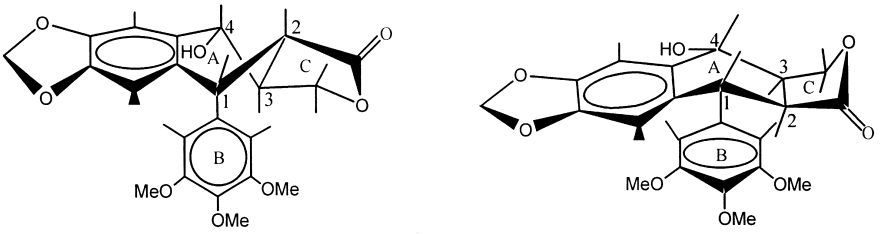


2 β -H configuration 2 α -H configuration

Fig. 1. Stereostructures of podophyllotoxin (2β-H configuration) and picropodophyllin (2α-H configuration).

search for a new analytical method for ascertaining 2-H configurations of these diastereoisomers.

Capillary electrophoresis (CE) has gained acceptance as an alternative technique to liquid chromatography in separation science research because of its merits such as high resolution, small sample volumes, extraordinarily small buffer solution consumption and rapid separation [7–10]. In this study, seven pairs of diastereoisomers of podophyllotoxin lignans were successfully separated by micellar electrokinetic chromatography (MEKC) for the first time. The buffer system was 5 m*M* sodium tetraborate–20 m*M* $NaH_2PO_4$–120 m*M* sodium dodecylsulfate (SDS)–30% (v/v) 2-propanol (pH 6.5–7). The migration behaviors of the compounds with different types and concentrations of organic modifiers were studied. The method can be used to identify the purification of the lignans, monitor the configuration transformation, and determine the 2-H configurations of the spin-labeled derivatives.

## 2. Experimental

### *2.1. Equipment*

The separation was carried out using a Waters Quanta 4000 capillary electrophoresis apparatus (Waters Chromatography Division of Milford, MA, USA) with a positive power supply. The data acquisition was carried out with a Maxima820 Chromatograph Workstation. Uncoated fused-silica capillaries of 75 μm I.D. (Hebei Yongnian Capillary Factory, China) with a total length of 50 cm and detection length of 42.5 cm were used. A PHS-10A pH meter (Xiaoshan Instrument Factory, China) was employed for pH measurements.

### *2.2. Materials*

4′-Demethylpicropodophyllin (p2), 4′-demethylpodophyllotoxin (2), picropodophyllin (p1), podophyllotoxin (1), picropodophyllone (p3), podophyllotoxone (3), desoxypicropodophyllotoxin (p4); desoxypodophyllotoxin (4); picropodophyllic acid hydrazine (p5), podophyllic acid hydrazine (5), picropodophyllic acid-[4-(2,2,6,6-tetramethyl-1-piperidyloxy)]hydrazone (p6), podophyllic acid-[4-(2,2,6,6-tetramethyl-1-piperidyloxy)]hydrazone (6), picropodophyllic acid-[3-(2,2,5,5-tetramethyl-pyrroline-1-oxy)]semicarbazone (p7) and podophyllic acid-[3-(2,2,5,5-tetramethylpyrroline-1-oxy)]-semicarbazone (7) were obtained from the Organic Chemistry Research Laboratory, Department of Chemistry of Lanzhou University, China (see Fig. 2 for the structures). The physical constants and spectrometric data of these components coincided with those in the literature [11–13]. All other chemicals were of analytical grade and were purchased from Xi'an Reagent Company, China. Deionized water was used throughout.

### *2.3. Electrophoretic procedure*

New capillaries were conditioned by rinsing with 0.1 *M* sodium hydroxide for 10 min, water for 5 min, and then the run buffer for 10 min, in order. Between

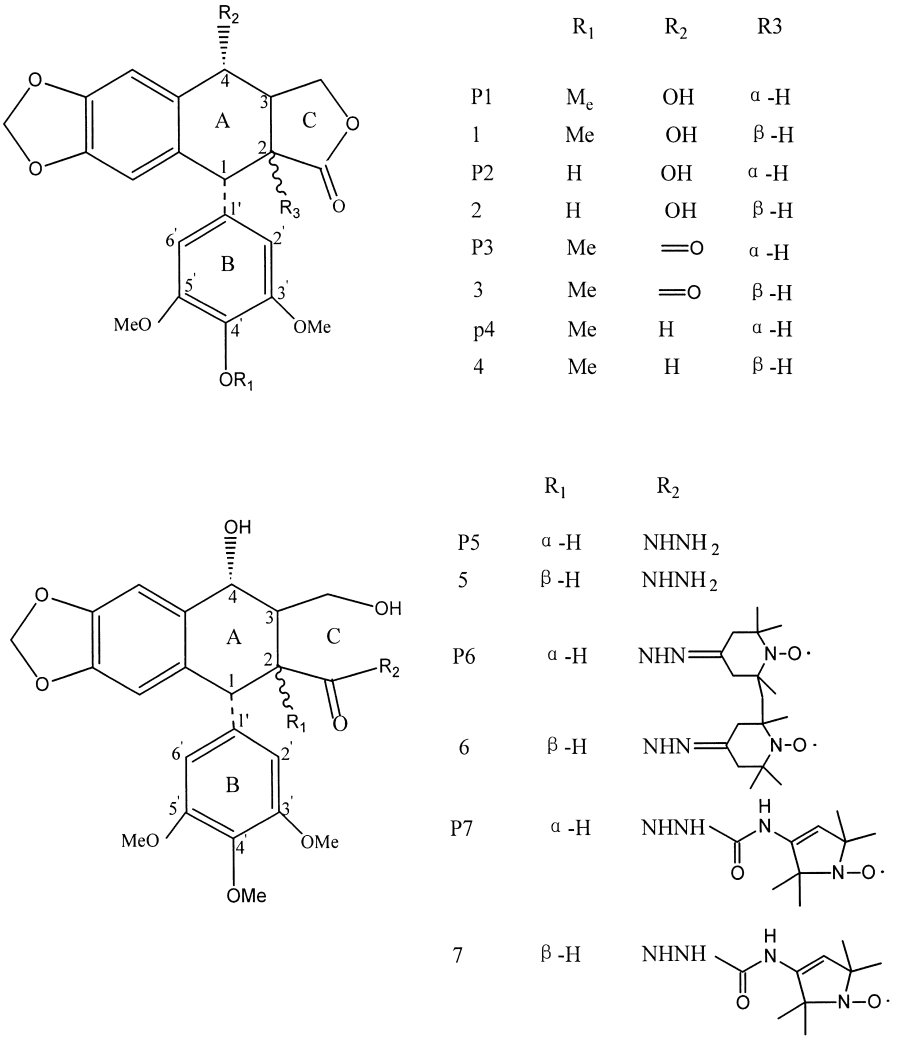


Fig. 2. Structures of seven pairs of diastereoisomers of podophyllum lignans. Compound identities shown in the Experimental section.

each run, the capillary was rinsed with water for 2 min, 0.1 *M* sodium hydroxide for 3 min, water for 2 min, and then the run buffer for 3 min, successively.

Stock solutions of 200 m*M* sodium dihydrogenphosphate, 200 m*M* SDS and 100 m*M* sodium tetraborate were prepared by dissolving the corresponding substances in water.

Micellar buffers were prepared by mixing 2.0 ml sodium dihydrogenphosphate stock solution, 1.0 ml sodium tetraborate stock solution and appropriate volume of SDS stock solution, then diluting to 20 ml. After the pH of the running buffer was adjusted with 0.2 *M* HCl or 0.2 *M* NaOH, an organic modifier was mixed into it. These electrolytes were filtered through a 0.45-μm membrane prior to use.

The temperature of the instrument was maintained at 25±0.5 °C with a laboratory-made temperature-controlled device. The detection wavelength was set at 214 nm and the voltage applied was 18 kV. Samples were injected in the hydrostatic mode at a height of 10 cm for 5 s.

Methanol solutions of about 100 μg/ml for the seven pairs of diastereoisomers were used in the analysis.

## 3. Results and discussion

For the separation of the seven pairs of diastereoisomers, it was reasonable to employ MEKC mode using SDS as the micelle-forming surfactant. Preliminary study indicated that electrolyte concentration slightly affected the separation. The optimization of the experiment was carried out by investigating the influences of SDS concentration, organic modifiers and the pH of run buffer on the migration times of the diastereoisomers.

### *3.1. Effect of SDS concentration*

On applying MEKC with SDS as micelle-forming agent, the separation was greatly improved. At low concentrations of SDS in the presence of 5 m*M* sodium tetraborate, 20 m*M* $NaH_2PO_4$ (pH 6.8) and 30% (v/v) 2-propanol, the peaks remained merged; following the increase in SDS concentration the micelles formed progressively and the diastereoisomers were separated in the migration order of p5, 5, p2, 2, p7, p6 (p1), 1, p3, 3, 7, 6, p4, 4. The sequence of p5 (5)>p2 (2)>p7>p6>p1 (1)>p3 (3)>p4 (4) were consistent with the polarity of the corresponding compounds. Perhaps due to the large difference in the steric structures between the two isomers in p6–6 and p7–7 pairs led by the large group $R_2$, 7 and 6 did not migrate immediately after p7 and p6, respectively, but after compounds 3 and 7, respectively.

At low concentrations of SDS, although p5–5, p6–6, p7–7 and p4–4 had been separated completely, p1–1, p2–2 and p3–3 still overlapped. For the resolution of all the seven pairs of diastereoisomers, 120 m*M* SDS was effective.

### *3.2. Effect of organic modifiers*

In MEKC, the addition of an organic modifier to the buffer contributes to the alteration of selectivity and improvement of resolution. In this study, the seven pairs of diastereoisomers heavily overlapped in the absence of an organic modifier and following the addition of methanol or 2-propanol, the separation was greatly improved, with the buffer containing 5 m*M* sodium tetraborate, 20 m*M* $NaH_2PO_4$ (pH 6.8) and 120 m*M* SDS (Fig. 3).

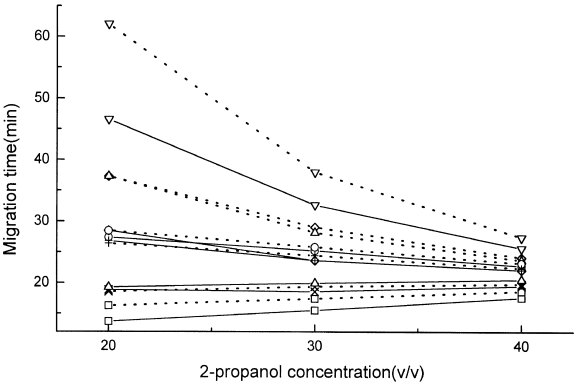


Fig. 3. Migration times versus 2-propanol volume percentage for the seven pairs of diastereoisomers. Electrophoretic conditions: 5 m*M* sodium tetraborate–20 m*M* $NaH_2PO_4$–120 m*M* SDS, pH 6.8, with various volume percentages of 2-propanol; +, (p1–1); ×, (p2–2); ○, (p3–3); ▽, (p4–4); □, (p5–5); ◇, (p6–6); △, (p7–7); solid line: 2α-H configuration; dotted line: 2β-H configuration. Compound identities shown in the Experimental section.

Due to the decrease in electroosmotic flow, the migration times of the five compounds p5, 5, p2, 2, p7 increased slightly with the concentration of 2-propanol. In contrast, the migration times of the others decreased with the percentage of 2-propanol. This might be attributed to the increased partition of the compounds into the aqueous phase.

With the exceptions of p1–1 and p2–2 pairs, the migration order of the two isomers in each of others pairs was always a 2α-H compound before its 2β-H isomer at any investigated concentration range of 2-propanol. In p1–1 or p2–2 pair, at 20% 2-propanol, a 2α-H compound migrated after its 2β-H isomer, whereas at 30–40% the order reversed. These phenomena could be explained as follows.

In each of the seven pairs of diastereoisomers, on the one hand, because a 2α-H compound exists with B ring in an equatorial conformation [12], it takes a more planar structure compared with its corresponding 2β-H isomer (Fig. 1). Thus the 2α-H isomer should be more readily solubilized to the micelles in the buffer solution [14]. On the other hand, due to the conformational flexibility of the 2α-H compound [12], which is able to arrange itself in conformation allowing for maximum interaction of its polar groups with an organic modifier [15], its partition into the aqueous phase should be higher than that of its corresponding rigid, inflexible 2β-H

isomer where molecular movement is restricted. In the MEKC running process, the two opposite tendencies competed with each other. If the effect of SDS on the migration behaviors of a pair of diastereoisomers outweighs that of an organic modifier, the 2α-H compound will migrate after its corresponding isomer; if the effect of an organic modifier prevails, the migration order will reverse. For p3–3, p4–4, p5–5, p6–6 and p7–7 pairs, the effect of 2-propanol was always dominant at the concentration range investigated, and thus the migration order of the two isomers in each of these pairs was always a 2α-H compound before its corresponding 2β-H isomer. For p1–1 and p2–2, at low concentrations of 2-propanol, the dominant effect of SDS on the migration behaviors led to a 2α-H isomer after its 2β-H isomer; at high concentrations of 2-propanol, the effect of the organic modifier outweighed that of SDS, therefore the order reversed.

With methanol as a modifier, the migration times of p5, 5, p7, p2, and 2 deceased little with the concentration of methanol, while the migration times of p1, 1, p3, 3, p4, 4, p6, 6 and 7 increased. The migration orders of the two isomers in p3–3, p4–4, p5–5, p6–6 and p7–7 pairs were the same as those with 2-propanol. These might be understood with a similar reason as discussed above. However, the order of the two isomers in p1–1 and p2–2 pairs was always p1 after 1, p2 after 2, respectively, not changing with the concentration of methanol. This indicated that the effect of SDS on the migration behaviors of the four compounds was stronger than that of methanol.

With methanol as a modifier, although each of the seven pairs of diastereoisomers were separated very well, p7 and 2 as well as p6 and 3 at 20%, or 4 and 6 as well as p6 and p3 at 30–40% merged together. In addition, the analysis time was very long. The optimum of the overall resolution for the 14 compounds was situated at 30% 2-propanol.

### *3.3. Effect of pH*

It was reported that the range of buffer pH in MEKC mode is usually from 6 to 9 [16]. If pH of the running buffer was over 8, the epimerization from 2β-H isomers to 2α-H isomers could occur in this experiment [17]. Thus the reasonable pH range of this study should be from 6 to 8. From the experiment results, it was found that the effect of pH on the resolution was less important than that of SDS-concentration or the organic modifiers. Due to the long analysis time at pH under 6.5 and the decreased resolutions at pH over 7, the optimum pH range was from 6.5 to 7.

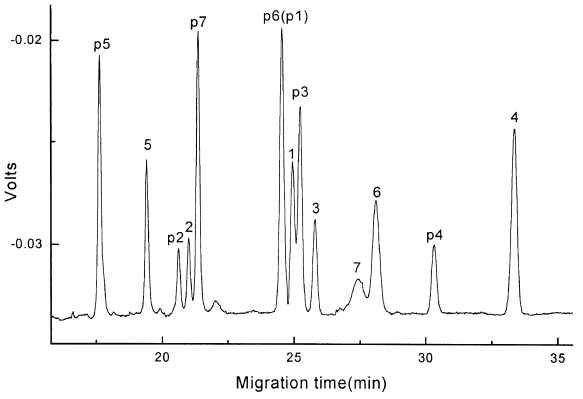


Fig. 4. Electropherogram for the separation of the seven pairs of diastereoisomers. Electrophoretic conditions: 5 m*M* sodium tetraborate–20 m*M* $NaH_2PO_4$–20 m*M* SDS–30% 2-propanol, pH 7.0; compound identities shown in the Experimental section.

After the effect of each single parameter on migration times had been studied, the measurements were carried out by combining the optimum values of each parameter, viz., 120 m*M* SDS, 30% 2-propanol and pH 6.5–7. The resulting electropherogram is showed in Fig. 4. Except for p6 and p1, the seven pairs of diastereoisomers were separated from each other within 35 min. It is interesting to note that every 2α-H compound migrated before its corresponding 2β-H isomer under these optimum conditions.

A study to test the repeatability of the separation for the electrophoretic system was performed. For a given sample, RSD-values of the migration times for five replicate injections were below 2.7%.

## 4. Conclusion

Seven pairs of diastereoisomers of podophyllum lignans at 2-position were firstly successfully separated by MEKC with 5 m*M* sodium tetraborate–20 m*M* $NaH_2PO_4$–120 m*M* SDS–30% (v/v) 2-pro-

panol (pH 6.5–7). The method developed can be used to identify the purification of the lignans, monitor the epimerization of 2-H in the isolation of natural products, chemical reactions and pharmaceutical metabolism, etc. It is especially significant that the method can be used to determine the 2-H configurations of the two pairs of spin-labeled compounds. According to the rule of the migration order of every pair of diastereoisomers under these optimum conditions, 2-H configurations of novel and analogous spin-labeled derivatives of podophyllum lignans could be predicted.

## References

[1] H.F. Stähelin, A.V. Wartburg, Cancer Res. 51 (1991) 5.
[2] L. Schacter, Semin. Oncol. 23 (Suppl. 13) (1996) 1.
[3] I. Jardine, in: J.M. Cassidy, J.D. Douros (Eds.), Anticancer Agents Based on Natural Products Models. Academic Press, London, 1980, p. 319.
[4] Y. Aso, Y. Hayashi, S. Yoshioka, Y. Takeda, Y. Kita, Y. Nishimura, Y. Arata, Chem. Pharm. Bull. 37 (1989) 422.
[5] X. Tian, Y.G. Wang, M.G. Yang, Y.Z. Chen, Life Sci. 60 (1997) 511.
[6] P. Kapf, K. Murokuma, R. Kreilick, J. Chem. Phys. 54 (1971) 105.
[7] R.S. Rush, A.S. Cohen, B.L. Karger, Anal. Chem. 63 (1991) 1346.
[8] A.R. Timerbaev, W. Buchberger, J. Chromatogr. A 834 (1999) 117.
[9] G. Gubitz, M.G. Schmid, Electrophoresis 21 (2000) 4112.
[10] A. Guttman, K.J. Ulfelder, Adv. Chromatogr. 38 (1998) 301.
[11] I. Jardire, R.J. Strife, J. Kozlowski, J. Med. Chem. 25 (1982) 1077.
[12] C.F. Brewer, J.D. Loike, S.B. Horwitz, J. Med. Chem. 22 (1979) 215.
[13] L. Yang, P. Liu, X. Tian, S. Hua, J. Li, Y. Chen, J. Lanzhou Univ. 20 (1984) 92.
[14] S.W. Sun, S.S. Lee, L.Y. Chen, C.K. Chen, J. Chromatogr. A 767 (1997) 277.
[15] C.K. Lim, D.C. Ayres, J. Chromatogr. 255 (1983) 247.
[16] Y. Fu, J. Lu, Capillary Electrophoresis, ZheJiang University Press, China, 1st ed., 1997, p. 95.
[17] J. Li, W. Zhao, J. Qu, Y. Su, Y. Chen, J. Lanzhou Univ. 24 (1988) 78.